Gastrointestinal & Digestive System

Raghavendra, J Gastrointest Dig Syst 2014, 4:6
http://dx.doi.org/10.4172/2161-069X.1000232

Research Article Open Access

Thrombophilia Evaluation during Acute Mesenteric Ischaemia

Raghavendra N*

Manipal Hospital, Surgical Gastroenterology Bangalore, Karnataka, India

***Corresponding author:** Raghavendra N, Associate consultant, Manipal Hospital, Surgical Gastroenterology Bangalore, Karnataka, India, Tel: 919535155044; E-mail: rags_dr@yahoo.co.in

Received date: June 24, 2014, **Accepted date:** Oct 27, 2014, **Published date:** Oct 31, 2014

Abstract

Background: Unlike studies on vascular thromboses in the limbs, there have been few studies in Acute mesenteric ischaemia (AMI) evaluating thrombophilia. We have attempted to analyze the usefulness of evaluation for heritable thrombophilia in AMI.

Methods: We retrospectively studied 29 patients with AMI. All patients underwent haematological tests to detect a deficiency of natural anticoagulant proteins. Sixteen of these patients underwent genetic studies for common genetic mutations accounting for heritable thrombophilia and other blood tests for the presence of procoagulant factors.

Results: Fifteen patients had mesenteric venous thrombosis (MVT) and 14 had mesenteric arterial occlusion (MAO). Of the 16 patients undergoing genetic studies, 7 (44%) were found to have MTHFR C677T polymorphism, none were found to have FVL and PT gene mutations. Protein C, S and AT III levels were decreased in most of the patients. The median levels of these were higher in patients with MVT than in those with MAO.

Conclusions: The MTHFR C677T polymorphism appears to be the most common genetic defect associated with AMI in India. Protein C, S and AT III levels measured during the acute thrombotic episode may not represent their actual levels to detect a deficiency.

Keywords: Thrombophilia; Acute mesenteric ischaemia; Anticoagulants; Thromboembolism

Introduction

Acute mesenteric ischaemia (AMI) remains a disease with high mortality even if diagnosed early [1]. Majority of the studies have focused on its modes of presentation or prognostic factors [2] but there have been few studies on thrombophilia in these patients. Most of our understanding on thrombophilia is from investigations on vascular thromboses involving the limbs. There is confusion regarding the usefulness of thrombophilia investigation during an acute event of thrombosis because of consumption of coagulation factors [3]. It would be unwise to discontinue anticoagulation even temporarily to investigate the cause of thrombosis as this might precipitate another episode of life threatening mesenteric vascular occlusion. Aware of these constraints we analyzed the presence of heritable thrombophilia in Indian patients with AMI to define its role in evaluating acute mesenteric vascular thrombosis.

Patients and Methods

We retrospectively studied 29 patients with AMI who presented to us between March 2010 to June 2012. They were identified from the departmental operative and discharge database which is recorded prospectively. We analyzed their clinical presentation, demographic variables, investigations at presentation and outcome. The diagnosis of acute mesenteric ischaemia before treatment was confirmed on CT angiography of abdomen.

Sixteen of these patients underwent genetic studies for common mutations accounting for heritable thrombophilia [Factor V leiden A506G (FVL), Prothrombin 20210A mutation (PT), Methylenetetrahydrofolatereductase C677T polymorphism (MTHFR)]. These were done by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. The results were reported as either positive or negative for the mutation tested and if positive, they were reported as either homozygous or heterozygous. The same patients underwent blood tests to detect presence of procoagulant factors [anticardiolipin antibodies, homocysteine and activated protein C resistance (APCR)]. The results of tests for lupus anticoagulant screen and anticardiolipin antibodies were reported as positive or negative.

All patients (n 29) underwent haematological tests to detect a deficiency of natural anticoagulant proteins [Protein C, S and Antithrombin III (AT III)]. Protein C and AT III levels were measured by automated chromogenic assay. Protein S was measured by automated latex ligand immuno assay. The results of anticoagulant proteins were expressed as percentage of normal levels. The normal value for protein C level was 70-140%, for protein S was 64-129% and was 85-120% for AT III.

The blood for thrombophilia investigation in these patients was collected at admission/ at diagnosis/ in the immediate postoperative period but, before starting anticoagulation in all the patients. We excluded 11 patients who were started on anticoagulants before referring to our unit.

The continuous variables were expressed as median (range) and categorical variables as number (percentage). The continuous variables were analyzed with Mann- Whitney U test and categorical variables with Fisher's exact test. Statistical analysis was done using GraphPad Software, Inc (USA).

Results

There were 22 males and 7 females whose median age was 52 years (range 25-78 years). Fifteen patients had mesenteric venous thrombosis (MVT) and 14 had mesenteric arterial occlusion (MAO). Twenty-six of them underwent surgery and 3 were managed conservatively (all due to venous thrombosis - all survived). Ten patients died – 3 with MVT (23%) and 7 with MAO (50%).

Patients with arterial disease were more likely to have risk factors of atherosclerosis (Diabetes, Hypertension) and had previous history of coronary artery or cerebrovascular disease, while the patients with venous disease had hypothyroidism more often and cirrhosis was found in two of them.

None of our patients had more than one site of thrombosis at presentation. Three patients had prior history of coronary artery disease, one had cerebrovascular disease and one had history of upper limb embolic event.

For the purpose of analysis we divided patients into those having MVT and MAO. There was no significant difference in the demographic factors and clinical presentation between the two groups except for the significantly shorter duration of symptoms in patients with MAO (2 vs 15 days; p 0.004). Patients with MAO had significantly longer length of bowel resected than those with MVT (198 vs 61 cm; p 0.003).

Genetic studies (Tables 1 and 2)

Of the 16 patients undergoing genetic studies, 8 had MVT and an equal number presented with MAO; 10 survived and 6 died. MTHFR C677T polymorphism was seen in 7 patients (44%), none were found to have FVL and PT gene mutations. Among these patients testing positive for MTHFR C677T, 6 were heterozygous and 1 was homozygous. Patients with MVT (6 of 8; 75%) were more likely to have this mutation than those with MAO (1 of 8; 13%)

Thrombophilia test	Abnormal/ significant result	MAO	MVT
Genetic studies (n 16)	7 (43.7%)	(n 8)	(n 8)
MTHFR C677T	0	1 (12.5%)	6* (75%)
FVL A506G	0		
PT20210A			
Haematological tests (n 29)	24 (83%)	(n 14)	(15)
Protein C	17 (59%)	14 (100%)	10 (66.7%)
Protein S	21 (72%)	11 (78.6%)	6 (40%)
Antithrombin III		11 (78.6%)	10 (66.7%)
Other blood tests (n 16)	6 (37.5%)	(n 8)	(n 8)
Homocysteine	1	4 (50%)	2 (25%)
APCR	5 (31.3%)	0	1
Lupus anticoagulant screen	1	2 (37.5%)	3 (25%)
Anticardiolipin antibodies		0	1

Table 1: Overview of test results and MAO vs MVT; *One patient had homozygous mutation of MTHFR C677T

SNo	Age	Sex	Cause	FVL	PT	MTHFR	Protein C	ProteinS	AT III	APCR	LAC screen	Homo-cystein	Comorbidity	Risk factors
1	75	M	Arterial	0	0	0	1	1	1	0	1	0	HTN	
2	64	M	,,	0	0	0	1	1	1	0	0	1		
3	52	F	,,	0	0	0	1	1	1	0	0	0	DM, HTN, CRF	
4	78	M	,,	0	0	0	1	1	1	0	0	1	DM, HTN, CVA	
5	62	M	,,	0	0	1	1	1	1	0	1	1	CAD	Surgery
6	55	M	,,	0	0	0	1	1	1	0	0	0	HTN, CAD	
7	63	M	,,	0	0	0	1	1	1	0	0	1	HTN	
8	49	M	,,	0	0	0	1	0	1	0	0	0	CAD	
9	57	F	,,				1	0	1					
10	46	M	,,				1	1	1				DM	
11	58	M	,,				1	1	0					
12	30	F	,,				1	1	0				DM	Past embolism
13	42	M	,,				1	0	1					
14	37	M	,,				1	1	0					

15	54	F	Venous	0	0	1	1	1	1	1	0	0	Hypothyroid	
16	62	F	,,	0	0	1	1	1	1	0	1	1	Hypothyroid, HTN	
17	60	M	,,	0	0	11	1	1	1	0	0	0	DM	
18	49	M	,,	0	0	1	0	0	0	0	0	0		
19	42	M	,,	0	0	1	1	0	1	0	1	0		Cirrhosis
20	57	M	,,	0	0	0	0	0	0	0	0	0	HTN	
21	52	M	,,	0	0	0	1	1	1	0	0	0	DM	Cirrhosis
22	42	M	,,	0	0	1	1	0	1	0	1	1		
23	40	F	,,				1	1	1					
24	46	M	,,				0	0	1					
25	56	M	,,				0	0	0					
26	32	M	,,				1	1	1					
27	32	M	,,				1	0	0					
28	52	F	,,				1	0	0				Hypothyroid	
29	25	M	,,				0	0	1					

Table 2: Description of abnormalities found on evaluation in each individual patient; *FVL- Factor V Leiden A506G, PT- Prothrombin 20210A, MTHFR- Methylene Tetrahydrofolate Reductase C677T, AT III- Antithrombin III, APCR- Activated protein C resistance, LAC- Lupus anticoagulant screen; 0- Normal test result, 1- Abnormal result (heterozygous mutation in genetic studies), 11- Homozygous mutation DM- Diabetes mellitus, HTN- Hypertension, CAD- Coronary artery disease, CVA- Cerebrovascular accident, CRF- Chronic renal failure

	MAO	**MVT**	**p Value**
Protein C (%)	**38.8**	**67**	**<0.0001**
Protein S (%)	51.2	70	0.05
Antithrombin III (%)	53.7	72.7	0.001
Homocysteine (mic mol/L)	24.9	14.6	0.94

Table 3: Comparison of patients with MAO vs MVT

Haematologic studies (Tables 1-4)

The blood levels of natural anticoagulants were found to be decreased in most of the patients. Protein C, S and antithrombin III levels were below the normal range in 24, 17 and 20 patients (89, 63 and 74 % respectively). In patients with MVT these were 67, 70 and 73 % of normal respectively, which were higher than those patients with MAO (39, 51 and 54 % respectively). The difference was statistically significant for Protein C and antithrombin III (<0.0001 and 0.001). The mean levels of Protein C, S and AT III levels were higher in those who survived (60, 68 and 61 % of normal) than in those who did not survive (47, 55 and 55% of normal), but the difference was not significant (Table 3).

	Survived	**Died**	**p value**
Protein C (%)	59.6	47.1	0.14
Protein S (%)	68.3	54.7	0.19
Antithrombin III (%)	61.4	55.3	0.71

Homocysteine (mic mol/L)	7.5	18.8	0.72

Table 4 : Comparison of pro/anti-coagulant levels in patients- Survived vs Died

Other blood tests for procoagulant factors (Tables 1 and 2)

Homocysteine levels were found to be raised in 6 patients (37.5 %), the level of increase was greater in patients with MAO than MVT (25 vs 15) and higher in patients who died than in those who survived (19 and 8 %), but the values were not statistically significant. We did not find any relation between MTHFR C677T mutation and serum homocysteine levels.

Lupus anticoagulant screen revealed positive result in 5 patients (3 with MAO and 2 with MVT). Anticardiolipin antibodies and APCR was positive in one patient each.

As is evident from the table 2, majority of our patients had abnormal values in more than one test evaluating thrombophilia.

Discussion

AMI is still a grave illness and is accompanied by a high mortality rate [2]. The majority of studies on AMI have focused on details of presentation and predictors of mortality, while investigations to evaluate thrombophilia in these patients have been rare.

As most blood tests done for evaluation of thrombophilia are not reliable when the patient is on anticoagulation [3], blood needs to be taken either in the acute phase before starting anticoagulation or after stopping anticoagulation once the patient is stable, at a time remote from acute event. The usual recommendation for thrombophilia evaluation is to postpone laboratory testing at least 3 months after an acute thrombotic episode, and for at least 2 weeks after discontinuation of oral anticoagulant therapy [4,5]. Blood tests done during acute event may be unreliable due to consumption of factors during thrombosis [3]. Discontinuation of anticoagulation for the purpose of evaluation may precipitate again an episode of life threatening mesenteric vascular thrombosis.

In the present study we have done thrombophilia testing in 29 patients with AMI (not just MVT), with a study period of about 2 years and have done the tests consistently at presentation to us before starting anticoagulants. The studies mentioned previously are not categorical about the timing of the investigation, as it can have profound effect on results. The genetic tests were done in only 16 patients due to financial reasons as the funding was from a grant and not borne by the patients. We found MTHFR C677T polymorphism (one homozygous and 6 heterozygous) to be the most common genetic defect (7 of 16; 43.8%) associated with AMI, which may or may not be causative. Although the heterozygous mutation is not considered a risk factor for thrombosis, we have found it to be the most common associated factor, if not causative. This is because Misra et al. [6] in their study on north Indian population have reported its prevalence in the general population to be 19%, while the reported rates in Western population ranges from 35- 53% [7,8]. The study population in the present study is a north Indian one and we have found higher rates of this polymorphism in our patients. In addition, we did not find any relation of MTHFR C677T polymorphism and serum homocysteine levels.

Most of our patients had abnormal low values of natural anticoagulants, which probably may not indicate their actual deficiency but a consequence of their consumption in acute thrombosis. We noticed that median levels of protein S, C and antithrombin III were lower in patients with MAO than those with MVT, but were statistically significant in the latter two.

Although the present study was carried out with a focussed intention of finding the relevance of thrombophilia evaluation in patients with AMI, limitations are many. The study population is not homogenous, study is retrospective with obvious limitations, test not being carried out in all patients and small number of study population to list a few.

Inherited hypercoagulable states have been reported to be present in up to 75% of patients with MVT [9-11]. FVL mutation is the most common cause as per the current world literature accounting for 20-40% of cases [12]. Resistance to activated protein C also occurs from other mutations in about 10 % of patients [13,14]. The PT mutation, with higher plasma prothrombin levels, is reported in 8 % of individuals with venous thrombosis, and in 2 % of healthy individuals [15]. In addition to these identifiable genetic causes, deficiencies of naturally occurring anticoagulant proteins- protein C, protein S, and AT III are the cause in about 8 to 10%, while antiphospholipid antibodies are present in approximately 4 % of patients [12,13,16,17].

Agaoglu et al. [18] in their study from Turkey on 28 patients with AMI (with comparison to 103 healthy individuals) found FVL mutation in significantly higher proportion of patients than controls and postulated that this might be significant predisposing factor in the pathogenesis of AMI. In a study from Europe, Amitrano et al. [11] found higher prevalence of FVL, PT G20210A and MTHFR TT677 mutations in patients with acute mesenteric venous thrombosis than controls.

Though most clinical studies have not found heterozygous mutation of MTHFR gene to be a causative factor in vascular thrombosis, there is some evidence that even heterozygous mutation leads to reduced enzyme activity in in-vitro studies [19]. The clinical studies have further shown that the serum level of homocysteine is more important as cause for vascular thromboses than the presence of this mutation, either homozygous or heterozygous [20,21].

Three Indian studies have specifically looked at the prothrombotic states in patients with acute mesenteric ischaemia/ splanchnic vessel thrombosis. Ahluwalia et al. [22] in their study on 13 patients with mesenteric vessel thrombosis over 10 years (11 venous and 2 arterial) found atleast one prothrombotic state to be present in about 62% of their patients, mainly protein S deficiency (31%), none positive for FVL mutation. In another study of 28 patients with MVT over 10 years from Western India, Amarapurkar et al. [23] reported prothrombotic conditions in 61% of patients, 25% with multiple etiology. They found protein C deficiency to be the commonest hereditary risk factor (26%) and FVL in 9% of patients. The study by Dutta et al. [24] included 36 patients with abdominal vein thrombosis, 20 with MVT. They found FVL mutation in 10% of their patients and have concluded acquired risk factors are more common in MVT. In contrast to the previous Indian studies, we did not find FVL mutation in any of our patients.

The reason may be small number of patients (16), particularly of those with MVT (8).

To the best of our knowledge no article in the literature has so far reported differences in the levels of natural anticoagulants in patients with arterial and venous disease during an acute episode of thrombosis. The reason for lower values in patients with MAO may be many. Patients with MAO had significantly longer median length of bowel gangrene than MVT (198 vs 61 cm), probably greater extent of thrombosis and sicker patient. The blood levels of these natural anticoagulants have been shown in different experimental [25] and clinical human studies to have prognostic value in sepsis, lower the initial value, severe the sepsis [26,27]. In addition, patients with MVT had a longer duration of symptoms than those with MAO before presenting to us, thereby giving them greater time for the levels of natural anticoagulants to recover (from low levels of acute thrombosis due to consumption). Also the levels of these proteins were lower in patients who died compared to those who survived, but the values were not statistically significant.

Because of these reports and results of our study, we believe that the levels of these natural anticoagulants probably indicate the disease severity and not their actual deficiency. In contrast to the usual perception that patients with venous thrombosis are more likely to have abnormal thrombophilia tests, in our study with tests being done during the phase of acute thrombosis, patients with arterial occlusion had more abnormal values in haematological tests than patients with MVT. Hence, the protein C, S and AT III levels may probably have a prognostic importance rather than aetiological role.

Conclusions

The MTHFR C677T polymorphism appears to be the most common genetic defect associated with AMI in India. Protein C, S and AT III levels measured during the acute thrombotic episode may not represent their actual levels to detect a deficiency but may correlate with the type of AMI, extent of bowel gangrene and severity of illness.

Acknowledgements

We would like to acknowledge the Gyan Burman Research Fund, Sir Ganga Ram Hospital, New Delhi for bearing the expenditure spent on blood tests for evaluating thrombophilia.

References

1. Mansour MA (1999) Management of acute mesenteric ischemia. Arch Surg 134: 328-330.
2. Schoots IG, Koffeman GI, Legemate DA, Levi M, van Gulik TM (2004) Systematic review of survival after acute mesenteric ischaemia according to disease aetiology. Br J Surg 91: 17-27.
3. Baglin T, Gray E, Greaves M, Hunt BJ, Keeling D, et al. (2010) Clinical guidelines for testing for heritable thrombophilia. Br J Haematol 149: 209-220.
4. Tripodi A, Mannucci PM (2001) Laboratory investigation of thrombophilia. Clin Chem 47: 1597-1606.
5. Tripodi A (2005) Issues concerning the laboratory investigation of inherited thrombophilia. Mol Diagn 9: 181-186.
6. Misra UK, Kalita J, Srivastava AK, Agarwal S (2010) MTHFR gene polymorphism and its relationship with plasma homocysteine and folate in a North Indian population. Biochem Genet 48: 229-235.
7. Adams M, Smith PD, Martin D, Thompson JR, Lodwick D, et al. (1996) Genetic analysis of thermolabile methylenetetrahydrofolate reductase as a risk factor for myocardial infarction. QJM 89: 437-444.
8. Anderson JL, King GJ, Thomson MJ, Todd M, Bair TL, et al. (1997) A mutation in the methylenetetrahydrofolate reductase gene is not associated with increased risk for coronary artery disease or myocardial infarction. J Am Coll Cardiol 30: 1206-1211.
9. Acosta S, Alhadad A, Svensson P, Ekberg O (2008) Epidemiology, risk and prognostic factors in mesenteric venous thrombosis. Br J Surg 95: 1245-1251.
10. Rhee RY, Gloviczki P (1997) Mesenteric venous thrombosis. Surg Clin North Am 77: 327-338.
11. Amitrano L, Brancaccio V, Guardascione MA, Margaglione M, Iannaccone L, et al. (2001) High prevalence of thrombophilic genotypes in patients with acute mesenteric vein thrombosis. Am J Gastroenterol 96: 146-149.
12. Thomas DP, Roberts HR (1997) Hypercoagulability in venous and arterial thrombosis. Ann Intern Med 126: 638-644.
13. Provan D, O'Shaughnessy DF (1999) Recent advances in haematology. BMJ 318: 991-994.
14. de Visser MC, Rosendaal FR, Bertina RM (1999) A reduced sensitivity for activated protein C in the absence of factor V Leiden increases the risk of venous thrombosis. Blood 93: 1271-1276.
15. Margaglione M, Brancaccio V, Giuliani N, D'Andrea G, Cappucci G, et al. (1998) Increased risk for venous thrombosis in carriers of the prothrombin G-->A20210 gene variant. Ann Intern Med 129: 89-93.
16. Martinelli I, Mannucci PM, De Stefano V, Taioli E, Rossi V, et al. (1998) Different risks of thrombosis in four coagulation defects associated with inherited thrombophilia: a study of 150 families. Blood 92: 2353-2358.
17. Heresbach D, Pagenault M, Gueret P, Crenn P, Heresbach-Le Berre N, et al. (1997) Leiden factor V mutation in four patients with small bowel infarctions. Gastroenterology 113: 322-325.
18. AÄŸaoÄŸlu N, TÃ¼rkyilmaz S, Ovali E, UÃ§ar F, AÄŸaoÄŸlu C (2005) Prevalence of prothrombotic abnormalities in patients with acute mesenteric ischemia. World J Surg 29: 1135-1138.
19. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, et al. (1995) A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. Nature Genetics 10: 111-113.
20. Ray JG, Shmorgun D, Chan WS (2002) Common C677T polymorphism of the methylenetetrahydrofolate reductase gene and the risk of venous thromboembolism: meta-analysis of 31 studies. Pathophysiol Haemost Thromb 32: 51-58.
21. Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, et al. (2002) MTHFR 677C-->T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA 288: 2023-2031.
22. Ahluwalia J, Agarwal C, Das R, Sachdeva MU, Wig JD, et al. (2011) Mesenteric vessel thrombosis and hypercoagulable states. Indian J Pathol Microbiol 54: 425-426.
23. Amarapurkar DN, Patel ND, Jatania J (2007) Primary mesenteric venous thrombosis: a study from western India. Indian J Gastroenterol 26: 113-117.
24. Dutta AK, Chacko A, George B, Joseph JA, Nair SC, et al. (2008) Risk factors of thrombosis in abdominal veins. World J Gastroenterol 14: 4518-4522.
25. Heuer JG, Sharma GR, Gerlitz B, Zhang T, Bailey DL, et al. (2004) Evaluation of protein C and other biomarkers as predictors of mortality in a rat cecal ligation and puncture model of sepsis. Crit Care Med 32: 1570-1578.
26. Fisher CJ Jr, Yan SB (2000) Protein C levels as a prognostic indicator of outcome in sepsis and related diseases. Crit Care Med 28: S49-56.
27. Pettilä V, Pentti J, Pettilä M, Takkunen O, Jousela I (2002) Predictive value of antithrombin III and serum C-reactive protein concentration in critically ill patients with suspected sepsis. Crit Care Med 30: 271-275.